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THE BEHAVIOUR OF CHROMATOLYSED MOTONEURONES STUDIED BY INTRACELLULAR RECORDING

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When the axon of a neurone is severed, the cell body exhibits a profound series of morphological and chemical changes (the axon-reaction or chromatolysis) that begins a few days later and reaches a maximum in 2–3 weeks (Cajal, 1909; Bielschowsky, 1932; Hydén, 1943; Nonidez, 1944; Bodian & Mellors, 1945; Brattgård, Edström & Hydén, 1957).

It has been shown that, concomitant with these morphological changes, chromatolysed motoneurones exhibit changes in their reflex responses (Downman, Eccles & McIntyre, 1953). At the height of the chromatolysis group I afferent volleys from muscles no longer evoked a monosynaptic reflex discharge having the characteristic features of brief latency and virtual synchrony. The reflex latency was lengthened by at least 0·5 msec, even during post-activation potentiation, and the reflex discharge was dispersed over many milliseconds. However, by testing for the facilitation of motoneurones, it was shown that there was still a synaptic excitation with the brief latency of a monosynaptic action, though it was subliminal for evoking a reflex discharge. These observations led Downman *et al.* (1953) to suggest that the monosynaptic activation of motoneurones was depressed during chromatolysis and that there had been a compensatory development of polysynaptic excitatory pathways.

These suggestions can be very effectively tested by the technique of intracellular recording. In a preliminary investigation Bradley, Brock & McIntyre (1955) reported that in chromatolysed motoneurones there was a normal latency for the excitatory post-synaptic potential (EPSP) generated by monosynaptic activation, but that it showed an abnormally prolonged and variable rising phase. This prolonged smooth rise of the EPSP accounted for the long

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latency of the spike discharge, which was initiated as soon as a critical level was attained. There was no evidence for the development of polysynaptic pathways. In other respects the chromatolysed motoneurones did not differ strikingly from normal. The present paper gives an account of a further investigation of chromatolysed motoneurones by intracellular recording and in part confirms Bradley *et al.* (1955). In addition, a study was made of the effects produced in these motoneurones by the application of currents through the intracellular electrode.

Recent investigations (Araki & Otani, 1955; Fatt, 1957; Fuortes, Frank & Becker, 1957; Coombs, Curtis & Eccles, 1957*a*, *b*) have shown that synaptic and direct stimulation of normal motoneurones causes an impulse to be generated in the initial segment (IS) of the motor axon (the axon hillock plus the non-myelinated axon), which has a much lower threshold than the soma-dendritic (SD) membrane. By employing the same methods of investigation it will now be demonstrated that the soma-dendritic membrane becomes much more excitable in the chromatolysed motoneurone, so that this important selective excitability of the initial segment is diminished or even lost. This finding in particular will be shown to account satisfactorily for the peculiarities of the reflex responses of chromatolysed motoneurones. A preliminary account has already been published (Eccles, Libet & Young, 1957).

METHODS

The preparation of cats with chromatolysing motoneurones was similar to that used by Downman *et al.* (1953). Either Lumbar 7, or Sacral 1 ventral root (L7 VR or S1 VR) was divided extradurally under aseptic conditions, and the cats were then studied at intervals of from 14 to 44 days post-operatively, mostly at 20–23 days. The micro-electrode technique for recording and for passage of direct current pulses across the cell membrane has been described previously (Brock, Coombs & Eccles, 1952; Coombs, Eccles & Fatt, 1955*a*). The micro-electrodes were usually filled with 0·6 M-K_2SO_4 in 1% agar, with a resistance of 10–20 MΩ, but in some 3 M-KCl was used. The operatively severed ventral root and an adjacent normal one were mounted individually on platinum electrodes for recording or for stimulating antidromically. Afferent volleys were delivered by stimulation of appropriate hind-limb nerves, and were recorded by an electrode making contact at the junction of the dorsal roots with the spinal cord. The cats were kept under light pentobarbitone sodium anaesthesia, and the spinal cord was transected at the first lumbar segment.

The identification of a given motoneurone as being a chromatolysing one was made by its antidromic response to stimulation of the operatively severed ventral root. The completeness of the operative transection of the root was checked post mortem, but could also be tested at the start of the experiment by observing whether a group I afferent volley set up any reflex discharge in the ventral root with a minimal latency similar to that for a normal ventral root, about 1·1 msec after arrival of the afferent volley at the cord, instead of about 1·6 msec, which is the minimum for discharge from chromatolysed motoneurones (Downman *et al.* 1953).

The identification of a given motoneurone with the specific muscle which it innervates obviously could not be made here by antidromic activation from the appropriate muscle nerve, as was done by Eccles, Eccles & Lundberg (1957*b*), since the ventral root had already been severed. It was assumed that a motoneurone belonged to the muscle whose group Ia afferents produced the

largest monosynaptic EPSP (excitatory post-synaptic potential). This is at least generally true for normal cells (Eccles, Eccles & Lundberg, 1957*b*). In any case, this identification was not critical for this investigation, and was only of significance when considering the question of the pattern of relative magnitudes of EPSP's obtained in response to afferent volleys arriving from different muscle nerves. Where the ventral root had been operatively severed, there was a small change in relative conduction velocities for group Ia as compared to group Ib afferents in those afferent fibres that accompanied the degenerating portions of the motor axons. This made it more difficult or impossible to secure a good separation in the arrival times of the two groups at the cord, although the lowest-threshold afferents were still apparently those of the Ia group.

With few exceptions the account given below is derived from the responses of chromatolysed motoneurones selected because they had a resting membrane potential in excess of – 50 mV, and a spike potential above 70 mV, in cats 14–27 days after ventral root section. Altogether about 200 chromatolysed motoneurones were studied in twenty separate experiments. The results in all these motoneurones were qualitatively similar to those selected on account of their larger responses. It should be noted that, although the input capacitance of the micro-electrode to earth was small (about 5 pF) it was sufficient to produce about 10% loss of amplitude for the spike potentials of the motoneurones (cf. Brock *et al.* 1952). It should also be added that potentials produced at the tip of the micro-electrode (cf. Adrian, 1956) would make the resting potential measurements a less reliable index of the cell's condition, following penetration by the micro-electrode, than was the magnitude of the spike potential.

RESULTS

The resting membrane potential and the action potential

In many chromatolysed motoneurones the resting membrane potential has been comparable with the potentials recorded in normal motoneurones (Brock *et al.* 1952; Coombs, Eccles & Fatt, 1955*a*). The mean value has been about −62 mV for a series selected on account of their large spike potentials (Appendix 1, column 3). However, in our experience with chromatolysed motoneurones a larger proportion of penetrations has yielded low values for both resting potential and spike potential, and deterioration has been more rapid than normal. It thus seems likely that chromatolysed motoneurones are more prone to injury by the penetrating micro-electrode.

Components of antidromic spike potential. Likewise the sizes of the spike potentials have often been comparable with those of normal motoneurones. A low spike potential has been regarded as the most significant evidence of neuronal damage, so with a few exceptions only motoneurones giving spike potentials above 70 mV have been chosen for illustration and discussion (Appendix 1, column 9; Appendix 2, column 4). Analysis of the spike potentials generated by propagation of antidromic impulses into chromatolysed motoneurones reveals that, as with normal motoneurones, they are composed of three all-or-nothing components, which are identifiable as the M spike (first myelinated segment), the IS spike (initial segment of motor axon) and the SD spike (soma-dendritic membrane) (cf. Coombs, Curtis & Eccles, 1957*a*).

Fig. 1 *A–C* shows a typical spike potential produced by an impulse propagating antidromically into a chromatolysed motoneurone. The electrically differentiated record of Fig. 1*A* gives a clear demonstration of the IS and SD

components, which begin at the two arrows, and it resembles the antidromic spike potential of normal motoneurones (Brock, Coombs & Eccles, 1953; Frank & Fuortes, 1956; Fatt, 1957; Fuortes *et al.* 1957; Coombs, Curtis & Eccles, 1957*a*, *b*). However, it differs quantitatively in three respects: the maximum slope of the IS spike potential is low, being only 0·23 of the SD slope (normal range, 0·30–0·6); the SD spike begins at a very brief interval after the onset of the IS spike—only 0·15 msec (normal range, 0·25–0·5 msec); the SD spike is initiated at a low level of depolarization—only 16 mV (normal range, 19–37 mV). The respective mean values for a series of chromatolysed motoneurones are 0·30, 0·14 msec and 14·5 mV (Appendix 1, columns 7, 6 and 8). Since the SD spike was almost always superimposed before the IS spike

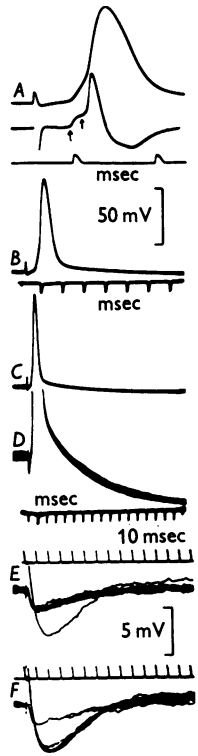

Fig. 1. Intracellular recording of spikes and after-potentials from chromatolysed motoneurones. Antidromically evoked response of a plantaris motoneurone (resting membrane potential about −60 mV) whose axon in L7 VR was severed 20 days previously. The antidromic spike potential in the upper trace of *A* is shown electrically differentiated in the lower trace, while *B* and *C* show the same response at progressively slower sweep speeds. *D* is at same speed as *C* (time scale below), but at about ten times higher amplification. *E* and *F* are at same amplification as *D*, but with a much slower sweep and are formed by superposition of about 20 faint traces. The stimulus was close to threshold for the motor axon, exciting it only once in *E* and in all but two traces of *F*.

reached its summit, the relative magnitude of the IS spike has been measured by the maximum slope of the rising phase, i.e. by the height of the differentiated record, rather than by its peak potential (Appendix 1, column 7).

The amplification of Fig. 1*A* was too small for the initial M spike to be recognized, but the M spike was visible when the IS–SD spike was abolished, as occurred in the second responses of Fig. 2*A*, *D*, *E*, and its onset is signalled by the first arrow in Fig. 2*F*. Usually the size of the M spike as recorded by an intrasomatic electrode was 2·5–4·5 mV (Appendix 1, column 4). In chromatolysed motoneurones it was certainly not abnormally small, as was the IS spike; rather was there a tendency for it to be larger than normal. In many chromatolysed motoneurones the interval between the beginnings of the M and IS spikes was within the normal range of 0·05–0·12 msec, but often it was longer, up to 0·22 msec (Appendix 1, column 5), which suggests a low safety factor for propagation and correlates with the relative frequency with which there was M–IS blockage of the antidromic impulse.

After-potentials. In Fig. 1*B*, *C* the spike potential is seen to decline on to a small after-depolarization, which decayed in a few milliseconds to an after-hyperpolarization, as may be well seen in the higher amplification of Fig. 1*D*. The full time course of the after-hyperpolarization is shown in Fig. 1*E*, *F* where the stimulus generating the antidromic impulse was just straddling the threshold for the motor axon. When it was usually just below threshold (*E*), it produced a hyperpolarization that has been shown to be due to an inhibitory post-synaptic potential set up by Renshaw cells activated through the motor-axon collaterals (Eccles, Fatt & Koketsu, 1954). When it was usually just above threshold (*F*), it produced a true after-hyperpolarization in addition. Note that, in contrast to the inhibitory potential, the after-hyperpolarization reversed after about 80 msec to a low depolarization. In respect of all these after-potentials the chromatolysed motoneurone does not differ significantly from normal.

Antidromic M–IS and IS–SD transmission. It was noted above that in chromatolysed motoneurones M–IS transmission tended to be delayed or blocked, and further evidence of a low safety factor for this transmission was provided by experimental procedures which impeded the propagation of the antidromic impulse into the motoneurone. One such procedure utilized the depression following a conditioning antidromic volley. For about 100 msec after an antidromic impulse has invaded a motoneurone, there is depression of the propagation of a second antidromic impulse due to refractoriness and the after-hyperpolarization (Brock *et al.* 1953). Normally M–IS transmission recovers within 2 msec, but a longer interval between volleys is required for IS–SD transmission, so that there is usually a considerable range of intervals during which the second antidromic impulse produces an IS spike, but no SD spike. In contrast, most chromatolysed motoneurones gave either an M spike

or a full M–IS–SD spike with the second impulse, as in Fig. 2*A*, *B*, *E*, *F*, there being no transitional phase of M–IS spike only. Thus with most chromatolysed motoneurones, there has been, at the critical interval for recovery of M–IS transmission, also recovery of IS–SD transmission (Appendix 1, columns 10 and 11). Usually recovery occurred at a test interval of less than 5 msec, but much longer recovery times were sometimes required. Fig. 2*C*, *D* also shows that similar behaviour occurred after conditioning by a spike potential fired by orthodromic stimulation. Thus, in summary, there are many instances in which the recovery time for the IS spike was longer than in normal cells, while the SD recovery times were in the normal range.

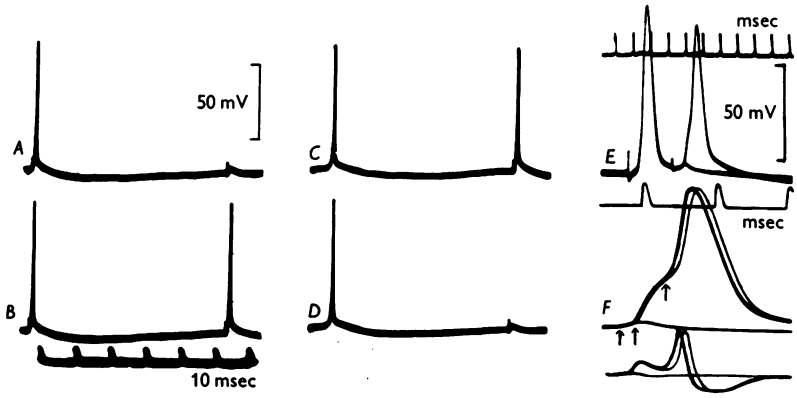

Fig. 2. Blockage of antidromic transmission by after-hyperpolarization and refractoriness. With the same cell as in Fig. 1 a second antidromic impulse was set up at a critical interval for transmission (55 msec) in the single sweeps *A* and *B*. Similarly, after synaptic activation of this same motoneurone (the first response in *C* and *D*) the same critical interval was revealed by the testing antidromic impulse. *E* and *F* (formed by superposition of several faint traces) show responses of another chromatolysed motoneurone (flexor digitorum longus, 20 days after section of its axon in L7 VR) to two antidromic impulses at a critical interval for invasion. In *F* the response to the second antidromic impulse is shown at a faster sweep with the electrically differentiated record below.

When depression of antidromic transmission was brought about by application of a hyperpolarizing current through the intracellular electrode, antidromic blockage also generally occurred in the M–IS stage of transmission into a chromatolysed motoneurone. Complementarily, when an antidromic impulse generated only an M spike at the normal membrane potential (Fig. 9*I*, *J*), the application of a depolarizing current restored transmission to IS and SD simultaneously (cf. Fig. 9*G*). These effects of applied currents were, however, often observed for normal motoneurones (Coombs, Eccles & Fatt, 1955*a*), and hence are not so discriminative as the depression following a conditioning antidromic impulse.

The excitatory and inhibitory post-synaptic potentials

It has been reported that group I afferent volleys cause chromatolysed motoneurones to give a reflex discharge which is dispersed temporally over several milliseconds and which has a minimum latency at least 0·5 msec longer than the normal monosynaptic reflex (Downman *et al.* 1953). These findings have been amply confirmed in the present investigation; hence it is of particular interest to investigate the synaptic potentials evoked by group I afferent volleys.

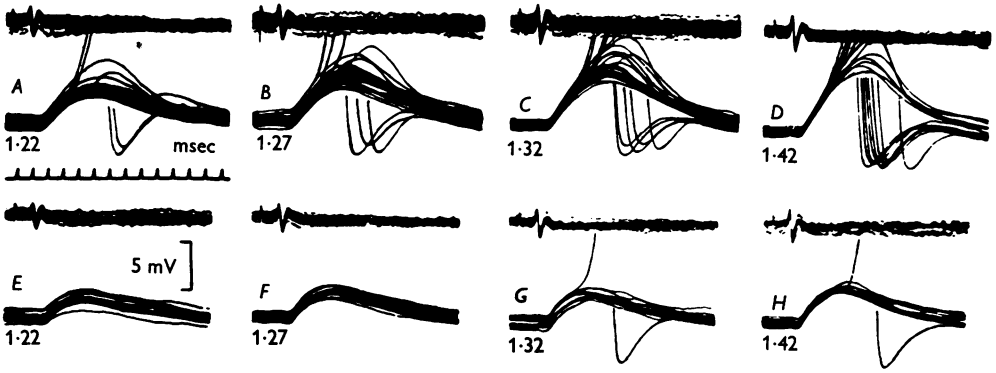

Fig. 3. EPSP's and partial responses. *Lower traces*: superimposed records, at about 5/sec for 5 sec, of intracellular responses of a biceps-semitendinosus motoneurone chromatolysed by severance of its axon in S 1 VR 16 days previously. *Upper traces*: triphasic action potentials recorded simultaneously by an electrode on the dorsal root entry zone at the lower L 7 level, negativity being recorded downwards. *A–H* are evoked by afferent volleys set up in biceps-semitendinosus nerve by stimuli at the indicated strengths relative to threshold. In *E–H* the motoneurone was hyperpolarized by application of a current of 5×10^{-9} A. The spike potentials of *A–D* and *G*, *H* have been in part retouched so as to recover losses in photography, but not so as to obscure the other records.

Monosynaptic excitatory action. Fig. 3 *A–D* shows the depolarizing potentials generated in a presumed biceps-semitendinosus motoneurone by group I afferent volleys in the biceps-semitendinosus nerve. These potentials show the same peculiarities that were reported by Bradley *et al.* (1955): slow and variable rate of rise and prolonged latency for initiation of impulses with a wide range of temporal dispersion. The impulses in Fig. 3 *D* arose, for example, after latencies of about 2·5–5·0 msec from the time of entry of the afferent volley into the spinal cord. In Fig. 8 *J* the range of latencies was much greater, 2·3–8·3 msec. The great variability of the successive depolarizing responses evoked by any one size of afferent volley is characteristic of chromatolysed motoneurones; this is in contrast with the regularly repeatable monosynaptic responses evoked by any particular group Ia volley in normal motoneurones (cf. Eccles, Eccles & Lundberg, 1957*a*, Figs. 2 and 4–8).

In agreement with Bradley *et al.* (1955) the depolarization was invariably initiated by the group I afferent volley with a brief latency within the normal range (0·5–0·7 msec) after its entry into the spinal cord (Appendix 2, column 5). Hence its first stage at least may be indentified as an excitatory post-synaptic potential (EPSP) set up monosynaptically. These monosynaptic EPSP's of chromatolysed motoneurones resemble those of normal motoneurones in that they are produced by the group of afferent fibres which have the lowest threshold to electrical stimulation and the fastest velocity, and which have been identified as the group Ia fibres from the annulospiral endings in muscle spindles (Laporte & Bessou, 1957; Eccles, Eccles & Lundberg 1957*a*). However, in the chromatolysed preparations it has only rarely been possible to distinguish between the group Ia and Ib afferent volleys by means of a differential conduction velocity, in the way that is possible in many normal preparations (Bradley & Eccles, 1953; Eccles, Eccles & Lundberg, 1957*a*; Laporte & Bessou, 1957).

Evidently in Figs. 3*A–D* some other depolarizing process was superimposed on the monosynaptic EPSP. Such a process is likely to be either an active spike-like response of some part of the motoneuronal membrane, which for present purposes will be termed a *partial response*, or an EPSP which has a longer latency because it is generated through a polysynaptic path (cf. Eccles, Eccles & Lundberg, 1957*c*). The 'partial responses' are likely to be due either to a full-spike activity of some discrete part of the motoneurone such as a dendrite, or to a local response arising in patches of the soma and adjacent dendrites that have their sodium conductance mechanism partially activated (cf. Hodgkin, 1951; Hodgkin & Huxley, 1952). In either case it may be assumed that they would be diminished or suppressed by any condition that caused a sufficient hyperpolarization of the motoneuronal membrane. On the other hand, EPSP's would be depressed little if at all under such conditions (Coombs, Eccles & Fatt, 1955*c*).

Effect of hyperpolarization on EPSP's and partial responses. This crucial test by hyperpolarization has been applied either by passing a steady hyperpolarizing current through the micro-electrode, preferably through one barrel of a double electrode, or during the after-hyperpolarization that follows a soma-dendritic spike potential. Invariably in chromatolysed motoneurones the synaptically evoked depolarizations have been greatly simplified by these procedures so that they have come to resemble normal EPSP's.

In Fig. 4*B* the application of a hyperpolarizing current of 5×10^{-9}A removed almost all the variable additions superimposed on the monosynaptic EPSP of Fig. 4*A*, and complete removal was produced by 10×10^{-9}A (Fig. 4*C*). On cessation of the current there was immediate restoration of the original type of response (Fig. 4*D*). A hyperpolarizing current of 5×10^{-9}A was similarly effective in Fig. 3*E–H*, while Fig. 5*E–K* shows a more compre-

hensive series with two different intensities of hyperpolarizing current, and afferent volleys set up by a range of stimulus strengths, the largest of which invariably generated full spike potentials. A hyperpolarizing current of 15×10^{-9} A was effective in suppressing all the additions to the monosynaptic EPSP's except occasionally with the responses to the largest afferent volley (Fig. 5*K*). In Fig. 6*B* the weakest hyperpolarization (4×10^{-9} A) suppressed the spike, but left the variable additions from which it arose in *A*. With 8×10^{-9} A in *C* these additions were diminished and delayed, while 10×10^{-9} A in *D* virtually cleared the EPSP of them.

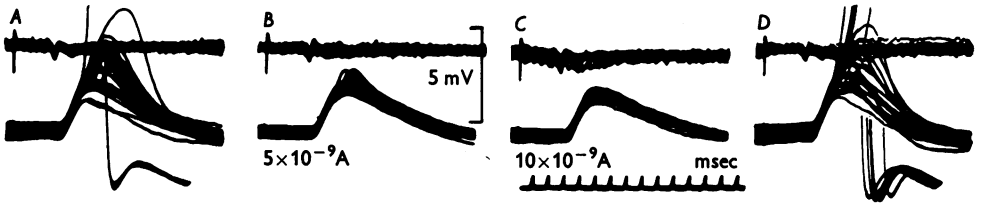

Fig. 4. Suppression of partial responses by hyperpolarization. Responses evoked by a gastrocnemius-soleus afferent volley in a gastrocnemius-soleus motoneurone (resting membrane potential about −60 mV) whose axon was severed in L7 VR 27 days previously. *B* and *C* were evoked by same stimulus as in *A* and *D* (about twice threshold), but during application of hyperpolarizing currents of 5 and 10×10^{-9} A respectively. Spike potentials have been retouched as in Fig. 3.

Similarly, in Fig. 5 *L–O* the after-hyperpolarization following an antidromically evoked spike potential was effective in suppressing all additions to the monosynaptic EPSP as in *A–D*, except in a few instances with the largest afferent volley.

It can therefore be concluded that in the chromatolysed motoneurone there is a large and variable addition of partial responses to the monosynaptic EPSP. When stripped of these partial responses, the remaining depolarizing potentials in general resemble normal EPSP's, but have a rather later summit. The normal interval to the summit of the monosynaptic EPSP ranged from 1·9 to 2·3 msec (Eccles, Eccles & Lundberg, 1957*a*, Figs. 4–10), though exceptionally the values were as brief as 1·2–1·4 msec, whereas in Figs. 3*E–H*, 4*C* and 5*I–K* the summits were respectively 3·0, 2·6 and 2·4 msec after the entry of the afferent volley into the spinal cord (cf. the values of Appendix 2, column 9). Possibly small local responses were still superimposed on the monosynaptic EPSP's on Fig. 3*E–H*, for example. Alternatively, there may be some addition of a later polysynaptic EPSP, or the time course of the monosynaptic EPSP may be somewhat slowed in chromatolysed motoneurones. It should be noted that the discrepancy is greater if the comparison is made, as it should be, with normal EPSP's during a comparable hyperpolarization, for the summit is then as much as 0·3 msec earlier (Coombs, Eccles & Fatt, 1955*c*) and the discrepancy would be in excess of 0·5 msec.

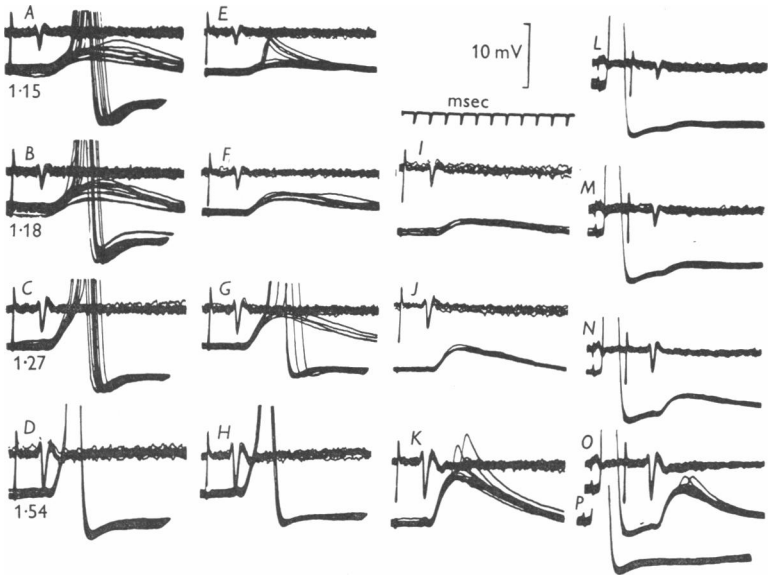

Fig. 5. Comparison of effects of hyperpolarization and after-hyperpolarization on spikes and partial responses. *A–K*, series as in Fig. 3, but for a gastrocnemius-soleus motoneurone (resting membrane potential about −50 mV) 16 days after severance of its axon in S1 VR. The stimulus strengths are given relative to threshold and were the same for all records in each row. Control responses for the various strengths are given in the first column (*A–D*), while the next two columns hyperpolarizing currents were passed through the intracellular electrode at the intensities of 4 and 15×10^{-9} A. In *L–O* the same afferent volleys, as with *A–D*, evoked responses during the after-hyperpolarization set up by an antidromic impulse, *P* being the control antidromic response. Same potential and time scales throughout. Spike potentials have been retouched as in Fig. 2.

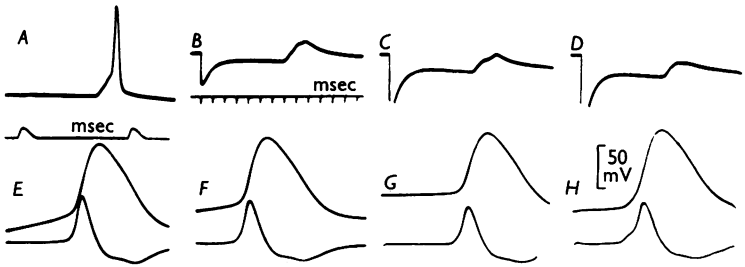

Fig. 6. Effects of applied currents on spike potentials and partial responses. Intracellular records from a plantaris motoneurone (resting membrane potential about −60 mV) chromatolysed by section of its axon in L7 VR 22 days previously. *A* shows control response to a plantaris afferent volley and in *B–D* hyperpolarizing currents of 4, 8 and 10×10^{-9} A were commenced, as shown, just after the onset of the trace. In the upper traces of *E–H* are shown the spike potentials evoked in this motoneurone by a plantaris afferent volley (*E*, *F*), by a depolarizing current of 14×10^{-9} A (*G*) and by an antidromic impulse (*H*). In F the plantaris afferent volley was superimposed on a background depolarizing current of 6×10^{-9} A. Lower traces in *E–H* are simultaneous records produced by electrical differentiation. Same potential scale throughout.

Sizes of EPSP's. In Figs. 3–6 the initial slope of the depolarizing potential was not appreciably affected when the hyperpolarizing currents were applied. With normal motoneurones hyperpolarizing currents of this order caused a relatively insignificant increase in the slope of the monosynaptic EPSP (Coombs, Eccles & Fatt, 1955*c*). It appears therefore that in the chromatolysed motoneurones this early steepest part of the depolarization is due to the monosynaptic EPSP and that the partial responses are superimposed somewhat later. This superposition is well illustrated in Fig. 4*A* and *D*, where there are a few responses resembling those of Fig. 4*C*. The maximum slopes of the monosynaptic EPSP's generated in chromatolysed motoneurones by homonymous afferent volleys have varied from 2 to 9 V/sec with a mean value of 5·5 V/sec (Appendix 2, column 6). These values are significantly lower than in normal motoneurones that have comparable resting and spike potentials, where the slopes of the monosynaptic EPSP's may be as high as 40 V/sec and are usually above 10 V/sec (cf. Coombs, Eccles & Fatt, 1955*c*; Eccles, Eccles & Lundberg, 1957*a*).

When the superimposed partial responses were removed by hyperpolarization, the sizes of the EPSP's were also significantly lower than normal. The slight prolongation of the rising time was far from compensatory for the abnormally low slopes of the rising phase. However, the possibility that the partial responses had not been entirely eliminated made the comparison of size less reliable than that of slope.

Time courses and sizes of the partial responses. The time courses of the partial responses that are superimposed on the EPSP's of chromatolysed motoneurones are approximately given by subtracting the underlying EPSP's that are observed during the application of a hyperpolarizing current. As seen in Figs. 3–5, the partial responses, as so determined, had a total duration of 4–6 msec and varied greatly in size, from less than 1 mV to more than 5 mV. Sometimes, as in Fig. 3*A–D*, the partial response had a rounded dome-like summit that was attained after a rising phase of 2–4 msec, as may also be seen in Figs. 4*A–D* and 5*A*, *B*. Alternatively, the partial responses had a brief rising phase and a sharp spike-like summit, as in Figs. 5*E*, *K* and *O* and 7*B*, *C*, *G*, and *I*. There were all transitions between the dome-like and spike-like partial responses. The dome-like response was always readily suppressed by a hyperpolarizing current (Figs. 3*E–H*, 4*C*), whereas spike-like responses sometimes persisted even with strong hyperpolarization (5*K*, *O*), and usually were suppressed in an all-or-nothing fashion. The probable loci of production of these two types of partial response will be considered later.

The inhibitory post-synaptic potential. In contrast to the complex and variable time course of the depolarizing responses produced by monosynaptic excitatory action, the inhibitory post-synaptic potential, IPSP, produced by direct inhibition, ran a time course that did not significantly differ from normal,

except perhaps in its somewhat more prolonged rising phase. For example, the IPSP set up by a quadriceps afferent volley in biceps-semitendinosus motoneurones had a latency varying from 1·4 to 1·9 msec, which was in the normal range, but thereafter the time to the summit was 1·2–2·0 msec (mean 1·55 msec), whereas it was about 1·1 msec with normal motoneurones (Coombs, Eccles & Fatt, 1955*b*, *d*). Thus there may be a comparable slowing of the rising phases of both the EPSP's and IPSP's of chromatolysed motoneurones. The investigation was not adequate to determine if there was any significant change in the size of the IPSP.

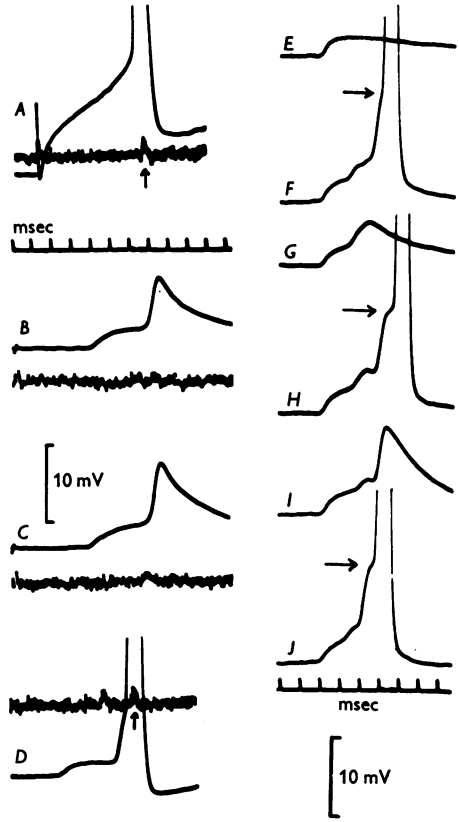

Fig. 7. Partial responses and the discharge of impulses. Upper tracings in *A–D* are intracellular records from a flexor digitorum longus motoneurone (spike potential, 84 mV) whose motor axon had been severed 16 days previously in L7 VR. Lower tracings are from the filament of L7 VR that contained the motor axon. *A* shows response to a depolarizing current pulse that generated a spike potential; *B–D* are responses at same amplification and sweep speed, but evoked by a group I maximum afferent volley in the nerve to flexor digitorum longus. *E–J* are responses as in *B–D*, which are selected to show the wide range of variability of partial responses that are superimposed on the EPSP (*E*) and evoke full spikes when the depolarization attains the critical level (about 13 mV) shown by the arrows in *F*, *H* and *J*.

The generation of impulses in chromatolysed motoneurones

It has been shown that, when normal motoneurones are stimulated synaptically or by a directly applied current, the impulse is generated in the initial segment, or possibly sometimes at the first node of the myelinated axon, and spreads thence down the motor axon and into the soma and dendrites (Araki & Otani, 1955; Fuortes *et al.* 1957; Coombs, Curtis & Eccles, 1957*a*, *b*). Unless the normal motoneurone is gravely deteriorated, each of the three components, the myelinated axon, the initial segment and the soma plus dendrites, gives an all-or-nothing spike response except in the very early stages of recovery from refractoriness. Thus in the generation of impulses the chromatolysed motoneurone differs from normal in two respects: partial responses were regularly observed with a synaptic stimulation that was inadequate to generate a full-size impulse; in response to monosynaptic stimulation the discharge of an impulse down the motor axon exhibited a very wide range of latency (cf. Fig. 8*A–C*). It appears from records such as those of Figs. 3 and 4 that the additional depolarization produced by the partial response caused the initiation of the spike potential. This is illustrated in detail in Fig. 7*F*, *H* and *J*, where a series of partial responses built up the depolarization to the level at which an impulse was generated at the arrows. Thus the wide range of latency of the partial responses would provide an explanation of the wide range of latency of the impulses discharged down the motor axons.

Partial responses and the discharge of impulses. Fig. 8*A–I* provides a particularly good illustration of the causal relationship of the partial responses to impulse generation, because the EPSP was extremely small, and a large partial response of practically constant time course and voltage (*I*) arose from it with a latency that varied by more than 1 msec (cf. Fig. 8*A–C* and the superimposed records of Fig. 8*H*). It should be noted that the identification of these depolarizing potentials as partial responses and not EPSP's depends on their wide range in latency (about 1 msec) and their occasional absence (cf. Fig. 8*D*, *H*), as well as on their brief time course. Except for the earliest spike of Fig. 8*H* the spike potential arose at approximately the same interval (0·5–0·7 msec) after the onset of the partial response. Fig. 8*A–D* shows typically the wide ranges of latency of the discharges in the ventral root fibres (cf. Downman *et al.* 1953), and it will be observed that the spike of the impaled motoneurone occurred at various times during the ventral root discharge, being relatively early in *A* and late in *C*, while it failed altogether in *D*. The ventral root spike potential produced by this motoneurone can be recorded in isolation by stimulating it directly (Fig. 8*E*), and so in the complex reflex discharges it can be identified as the spike potentials with summits marked by the arrows in Fig. 8*A*, *B*, and more doubtfully in *C* where another spike is superimposed on it. Thus, with direct and synaptic stimulation, these

ventral root spikes arose at approximately the same latency (0·34–0·38 msec) after the onset of the intrasomatically recorded spikes. This similarity of latency was more effectively illustrated in Fig. 9*A*, *C*, and *E* (the range being there 0·27 – 0·30 msec) because only one or two impulses were reflexly discharged into the ventral rootlet.

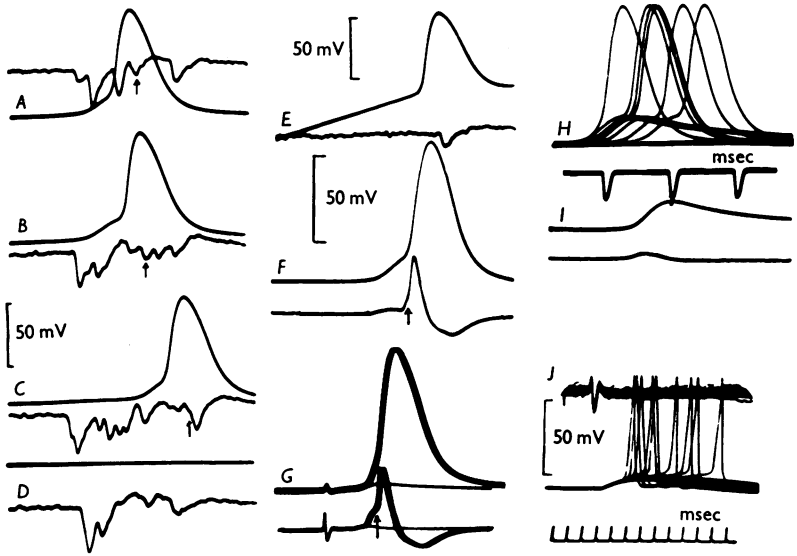

Fig. 8. Synaptic stimulation, partial responses and discharge of impulses. *A–D* and *F*, *H*, *I* were evoked in a gastrocnemius-soleus motoneurone (axon severed 21 days before in L 7 VR) by a maximal group I afferent volley in the gastrocnemius-soleus nerve, there being in *H* about 10 superimposed traces. *G* shows superimposed spike potentials evoked by an antidromic volley alone; *E* is the response evoked by application of a depolarizing pulse through the micro-electrode. The lower traces of *B–E* (upper of *A*) were recorded from the filament of L 7 VR that contained the axon of the motoneurone; the lower traces of *F*, *G* and *I* are the electrically differentiated records, the arrows in *F* and *G* indicating the onset of the SD spike. Same time scale for *A–I*, but *A–E* are at a lower amplification than the remainder (note potential scales). Note that, as shown in the upper tracing of *D* and some tracings of *H*, the local responses arise from an EPSP which is negligible at the low amplifications employed in this figure. *J* shows superimposed responses evoked in a plantaris motoneurone (14 days after axon section) by a plantaris afferent volley, as indicated in the upper trace. In *H* and *J* the spike potentials have been retouched.

The location of partial responses. When considering the region of the motoneurone that is responsible for generating the partial responses, it is of importance to determine if partial responses alone are ever responsible for the discharge of impulses down the motor axon. This could be tested either directly by recording from the ventral root, or indirectly by determining if partial responses were ever followed by a refractory period during which it was impossible to generate an antidromic impulse. Direct testing is illustrated in Fig. 7*A–D*. Stimulation by the application of a depolarizing current to the

soma in *A* revealed the configuration of the spike potential (indicated by arrow) in the ventral rootlet, and a similar potential is indicated by an arrow in *D*, where synaptic stimulation evoked a full-size spike as recorded intrasomatically. However *B* and *C* show typically that large spike-like partial responses did not cause the discharge of an impulse. Indirect testing invariably gave the same result, for partial responses never caused refractoriness in the motor axons. Since the partial responses have thus never been found to be directly responsible for evoking a discharge down the motor axon, it is improbable that they are produced in the initial segment, i.e. the axon-hillock and non-myelinated axon.

The spike-like partial responses with rising phases of less than 1 msec (Figs. 5*E*, *K* and *O*; 7*B*, *C*; 8*I*) most probably are generated in dendrites some distance away from the soma because usually they have been difficult to suppress with a hyperpolarizing current. A potential wave of this type would be expected if a propagating impulse were generated remotely in a dendrite, but failed to continue propagating down towards the soma. Such an origin would account also for the relatively constant size and time course that are usually observed for these partial responses in any one motoneurone. It would also account for the frequent absence of a good correlation between their incidence and the level of EPSP depolarization which is being recorded presumably within the soma.

The dome-like type of partial response was much more readily depressed by a hyperpolarizing current, so presumably it could be produced by partial responses of patches of the soma membrane or of the adjacent dendritic membrane. Its variability of size and time course (cf. Figs. 3*A–D*; 4*A*, *D*; 5*A*, *B*) may be ascribed to the variable growth and spread of partial responses arising in the excitable patches of the membrane. This type of partial response thus appears to resemble the local responses that have been investigated, particularly in axons. It will emerge in the Discussion that on other grounds it is also necessary to postulate that patches of the soma-dendritic membrane have an increased electrical excitability.

IS–SD transmission with synaptic and direct stimulation. With normal motoneurones it has been shown that the spike potentials generated by synaptic or direct stimulation had the same sequence of IS and SD spike potentials as with antidromic stimulation (Araki & Otani, 1955; Fatt, 1957; Fuortes *et al.* 1957; Coombs, Curtis & Eccles, 1957*a*, *b*). However, on account of the much briefer IS–SD interval, 0·14–0·25 msec for synaptic as against 0·25–0·5 msec for antidromic stimulation, differentiation of the spike potential was often necessary in order to reveal the inflexion between the IS and SD spikes. In the chromatolysed motoneurone it has already been seen that with antidromic stimulation the IS–SD interval was abnormally brief (0·10–0·18 msec). Comparison of the differentiated records of Fig. 9*B* with those of the

antidromic spike in *G* reveals that with direct stimulation of this motoneurone the IS spike potential slightly preceded (by 0·07 msec) the SD spike that arose at the arrow. However, no trace of an inflexion from IS to SD spike was seen with synaptic stimulation, as is shown typically in Fig. 9*D*, *F*. An absence of a clear IS–SD inflexion similarly occurred with the motoneurone illustrated in Fig. 6, both with orthodromic (*E*, *F*) and direct (*G*) stimulation, though it was evident with antidromic (*H*) stimulation, where the IS–SD interval was 0·13 msec. On the other hand, with some chromatolysed motoneurones a slight inflexion, presumably indicative of an IS–SD separation, could be seen in the differentiated records of spike potentials evoked by synaptic stimulation. For example, comparison of the differentiated records of the spike potentials in Fig. 8*F* and *G* reveals that in the synaptically evoked spike there was a slight

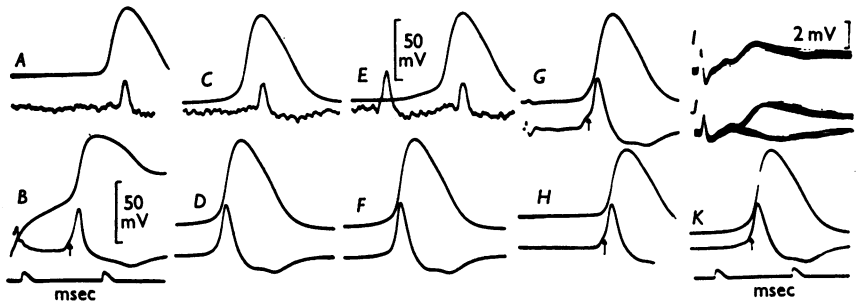

Fig. 9. Locus of initiation of spike potentials. Upper traces of *A–H* and *K* are intracellular records of spike potentials evoked in a gastrocnemius-soleus motoneurone (resting membrane potential about −68 mV) whose axon was severed in the L7 VR 23 days previously. In *A*, *B*, *H* the spike potentials were evoked by a depolarizing current pulse, in *C–F* and *K* by an afferent volley in gastrocnemius-soleus nerve and in *G* by an antidromic impulse when there was a background depolarizing current of 5×10^{-9} A. In *B*, *D*, *F–H* and *K* the lower tracing is the electrically differentiated record, the arrows in *B*, *G*, *H*, *K* indicating the onset of the SD spike, while in *A*, *C* and *E* the lower tracing was recorded from the filament of L7 VR that contained the motor axon. With records *I*, *J* (each with about forty superimposed traces) blockage of antidromic transmission occurred between M and IS in the absence of a background depolarization. In *J* the antidromic stimulus was just at threshold for the motor axon, exciting it on about half the occasions, while in *I* it was about 50% stronger. The M spike is identifiable as the difference between the two traces in *J*, while in *I* it can be seen to have moved earlier. Same time scale for all records, and same potential scale for all except *I* and *J*.

IS–SD separation like that shown in the antidromic spike, the respective IS–SD intervals, measured to the arrows, being 0·10 and 0·14 msec. Furthermore, there appeared to be a change in the response of the motoneurone illustrated in Fig. 9, because later (*K*) in the differentiated record a slight IS spike could be detected preceding the SD spike evoked by synaptic stimulation. The IS–SD interval as measured to the arrow was about 0·07 msec, while with direct stimulation (*H*) the IS–SD interval was lengthened to 0·11 msec.

Site of impulse generation. In attempting to discover the site at which direct and synaptic stimulation generated impulses in normal motoneurones, a comparison was made between the interval from the onset of the IS spike potential in the intrasomatic record to the onset of the corresponding spike potential in the ventral rootlet on the one hand, and on the other hand the conduction time for an antidromic impulse in the reverse direction over this same path (Coombs, Curtis & Eccles, 1957*b*). In this way it was shown that the impulse usually was generated in the myelinated axon by the earliest part of the IS spike potential, but with some motoneurones it appeared so early in the ventral root that a primary origin in the myelinated axon was presumed. However there was some uncertainty in the evaluation of the conduction time in the orthodromic direction because it was occurring under more favourable conditions than the antidromic conduction, e.g. in an axon that was already somewhat depolarized by the EPSP or by the directly applied current.

Similar investigations on chromatolysed motoneurones have shown that with direct and synaptic stimulation there was virtually the same orthodromic conduction time from the intrasomatic spike potential to the ventral root spike, but there was always a much longer antidromic conduction time, as measured between the delivery to the ventral root of a stimulus several times the axon threshold strength and the onset of the intrasomatically recorded IS spike potential. For example, in Fig. 8 the direct (*E*) and orthodromic (*A*, *B*) conduction times were 0·36–0·38 msec, while the antidromic time (*G*) was 0·59 msec, and similarly in Fig. 9 the respective times were 0·27–0·29 msec and 0·51 msec. In part this discrepancy can be attributed to the long M–IS interval that obtains for some chromatolysed motoneurones (cf. Appendix 1, column 5). When this factor was eliminated by measuring the antidromic conduction time to the onset of the M spike as in Fig. 9 *I*, it was shortened to 0·39 msec.

These values could be taken to indicate that, at least in some chromatolysed motoneurones, direct and synaptic stimulation generated the spike discharge initially in the myelinated axon. However, it should be remembered that, even with relatively strong stimuli, a few hundredths of a millisecond would be occupied in generating the antidromic impulse and that a considerable allowance should also be made for the effect of the preliminary depolarization in accelerating the conduction of the orthodromic impulse. Thus more information is required about these factors before a conclusion can be reached in regard to the precise site of initiation of the spike discharge.

Soma-dendritic origin of synaptically evoked impulses. Since in some motoneurones with intrasomatic recording it was impossible to demonstrate that, with the synaptically evoked spike potential, an IS spike preceded the SD spike (cf. Figs. 6, 9), it is likely that the spike was initiated in the soma-dendritic membrane before or simultaneously with its initiation in the initial segment of the motor axon, i.e. that the normal IS–SD sequence was even changed to SD–IS. Such an eventuality could not be tested with intrasomatic recording, for under such conditions the IS spike could be observed only if it preceded the SD spike by at least 0·05 msec. An IS spike arising simultaneously with the SD spike or a little later would not be detectable with intrasomatic recording. However, occasionally the micro-electrode was inserted into the initial segment (cf. Coombs, Curtis & Eccles, 1957*a*, *b*) and consequently gave opportunity for displaying the postulated SD–IS sequence.

For example in Fig. 10 the antidromic spike potential was the simple spike of 47 mV seen in the initial responses of *B* and *C*, while the synaptically evoked

spike potential of 60 mV (*A*) was shown by the differentiated record to be compounded of two spikes. The synaptically evoked spike was preceded by an EPSP of about 5 mV and was associated with the after-hyperpolarization (*K*) that typically follows a soma-dendritic spike (Coombs, Eccles & Fatt, 1955*a*). With the antidromic spike there was virtually no after-hyperpolarization (*L*); hence it may be assumed that the antidromic impulse did not invade the soma-dendritic membrane. When the synaptic stimulus was applied at progressively briefer intervals after the conditioning antidromic response, it is seen in

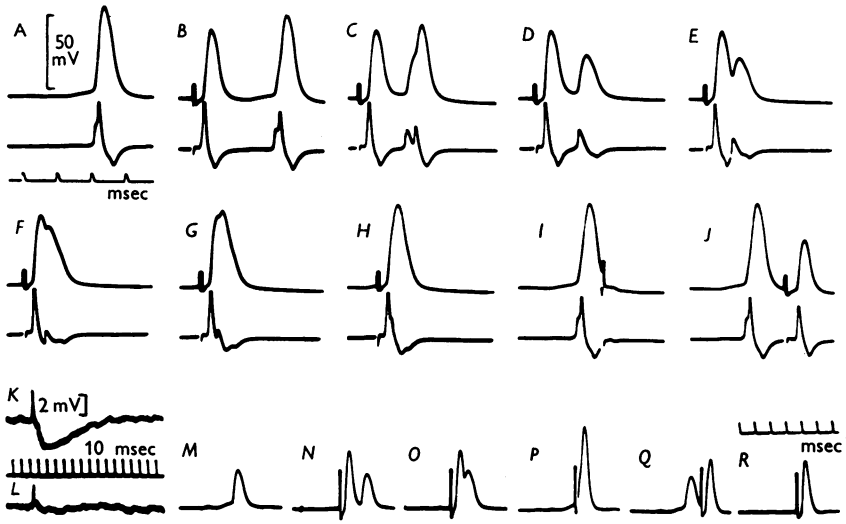

Fig. 10. Spike potentials of the initial segment. Responses recorded from a flexor digitorum longus motoneurone (resting membrane potential about −70 mV) by an intracellular microelectrode probably in the initial segment (see text), the motor axon in L7 VR being severed 22 days previously. Lower traces in *A–J* are the electrically differentiated records. *A* is control response evoked by a flexor digitorum longus volley alone; *B–J* are responses at same amplification and speed to an afferent volley and an antidromic impulse at various relative intervals, the antidromic response leading in *B–H*. In *I* the antidromic stimulus was applied after the synaptically evoked impulse and failed to evoke an antidromic impulse. *K* and *L* are at much higher amplification and slower sweep speed showing after-potentials following spikes evoked by synaptic and antidromic stimulation respectively. *M–R* are later responses of same motoneurone, *M* to the afferent volley and *R* to the antidromic impulse, while both are at various relative intervals in *N–Q*. Note slower sweep speed. Further description in text.

Fig. 10*B–H* that the second component of the synaptically evoked spike potential became delayed (*B*, *C*) and then suppressed, while the initial phase was virtually unaffected and was even (*H*) superimposed on the antidromic spike to give a spike that had virtually the same size as the control of the synaptically evoked spike (*A*). Evidently the second component of *A* involved the same membrane as the sole component of the antidromic spike, and with svnaptic stimulation this membrane was excited secondarily to the soma-

dendritic membrane, but gave a larger recorded spike potential, as would be expected if this spike was generated in the initial segment and the micro-electrode was inserted therein (cf. Coombs, Curtis & Eccles, 1957*a*). Thus it is assumed that there was IS–SD blockage of the antidromic impulse, but SD–IS transmission occurred for the synaptically evoked impulse.

This interpretation is supported by the finding that the motor axon was rendered refractory for a brief interval after the synaptically evoked spike (*I* and *J*), and by the series of records (*M–R*) as the motoneurone was deteriorating following withdrawal and reinsertion of the micro-electrode. The synaptic stimulus then evoked the small spike (Fig. 10*M*), which was still associated with an after-hyperpolarization, but was now failing to propagate to the axon, while the antidromic stimulus gave the larger simple spike (*R*). These two all-or-nothing spikes evidently were produced in quite independent areas of membrane, for they could be superimposed at all intervals (*N–Q*) giving a large summed spike (*P*) when virtually synchronous.

Thus Fig. 10 shows that the synaptic stimulus caused an impulse to arise primarily in the soma-dendritic membrane, while the initial segment responded secondarily about 0·1 msec later. It could be argued that this anomalous behaviour is attributable to the damage which the micro-electrode inflicted on the initial segment, and records *M–R* certainly show that the damage was then extensive enough to block transmission in either direction between the initial segment and the soma-dendritic membrane. However, the observations of Fig. 10 do suggest that the synaptically evoked impulse was primarily generated in the soma-dendritic membrane in those motoneurones where no initial IS spike could be observed (cf. Figs. 6, 9). In other chromatolysed motoneurones (Fig. 8) an initial IS spike could be detected, but even then the very brief IS–SD interval indicated that the soma-dendritic membrane had an abnormally low threshold.

Synaptic excitations from different afferent sources. When motoneurones were monosynaptically excited by afferent volleys from several different sources, there were sometimes very different threshold levels at which the observed EPSP's caused the initiation of impulses. For example in the motoneurone (probably plantaris) illustrated in Fig. 11*A–C*, the EPSP produced by a plantaris volley was almost always effective at a depolarization of about 1 mV (*B*), while a gastrocnemius volley was frequently effective at a negligibly small depolarization (*C*), and a depolarization of about 1 mV was always ineffective when produced by a flexor digitorum longus volley (*A*). A comparable series with a motoneurone from another experiment is illustrated in Fig. 11*E–G*, where the gastrocnemius volley was also specially effective (*G*) in generating an impulse despite the very low depolarization that it produced. Presumably in both these cases the gastrocnemius volley was effective because it generated the impulse in some focus that was so remotely placed on a

dendrite that it caused very little depolarization in the soma unless it propagated right through it and so down the axon. In the first series a hyperpolarizing current reduced the spike potentials of *C* to spike-like partial responses (*D*), which further indicates that the impulse was arising in a remote dendritic area and propagating thence through the soma, unless it was blocked by a hyperpolarizing current.

Responses of the type illustrated in Fig. 11 are to be expected occasionally if there are special strategic groupings of synapses on dendrites and if impulses are generated primarily in the SD membrane and not, as normally, in the IS membrane. In this latter situation it would be expected that all synaptically induced depolarizations would be effective in generating an impulse at much the same threshold level, as is observed normally (Coombs, Curtis & Eccles, 1957*b*).

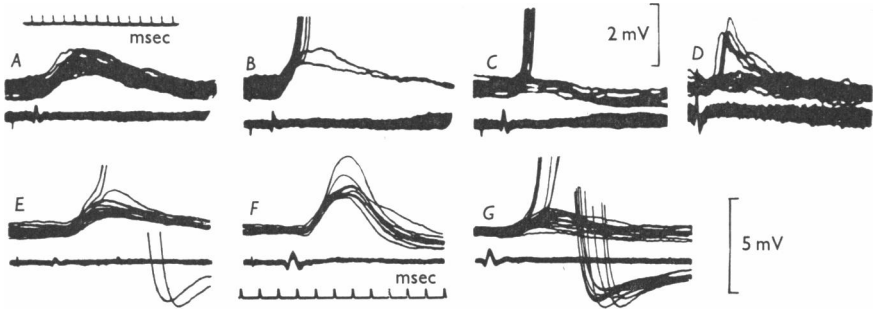

Fig. 11. Synaptic excitations from different afferent sources. Records as in Fig. 3. of two motoneurones, *A–D* and *E–G*, assumed to be plantaris, with axons severed 20 and 44 days previously. *A*, *B* and *C* show respectively the responses evoked by afferent volleys in flexor digitorum longus, plantaris and gastrocnemius-soleus nerves. *D* is evoked as in *C* but there was in addition a background hyperpolarizing current of 7×10^{-9} A. In *E–G* the responses were also evoked by afferent volleys in flexor digitorum longus, plantaris and gastrocnemius-soleus nerves respectively. Spike potentials have been retouched as in Fig. 2.

Some electrical properties of the motoneurone membrane

In some experiments double-barrelled electrodes were inserted into motoneurones so that currents could be passed through one barrel and the potential recorded through the other barrel (Coombs, Eccles & Fatt, 1955*a*; Coombs, Curtis & Eccles, 1956, and unpublished observations). A compensatory circuit has been used in order to minimize artifacts due to capacitive and resistive coupling (Coombs, Curtis & Eccles, unpublished observations.) In this way it was possible to determine the time course of the potential change produced across the motoneuronal membrane by rectangular pulses of current.

For example, in Fig. 12*A* the upper trace gives the recorded potential change when a depolarizing current of 4×10^{-9} A was passed through one barrel of a micro-electrode in an intracellular position, while with the lower trace the

same current was passed but the micro-electrode had been withdrawn to a just extracellular position, the setting of the compensatory circuit being unaltered. The difference between the two curves gives approximately the time course of the potential change across the motoneuronal membrane. Similarly, with Fig. 12*B–D*, the applied rectangular currents were depolarizing, 2×10^{-9} A and hyperpolarizing, 2×10^{-9} A and 4×10^{-9} A respectively. However, the

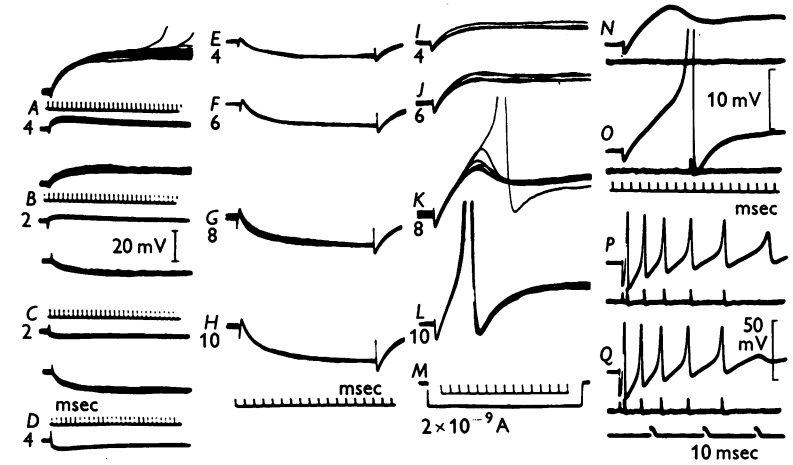

Fig. 12. Electrical properties of chromatolysed motoneurone. Responses produced by the application of current pulses to a biceps-semitendinosus motoneurone *A–D* with resting membrane potential about −60 mV (23 days chromatolysis) and to an unidentified motoneurone (*E–Q*) with resting membrane potential of about −50 mV (16 days chromatolysis). In each series the double micro-electrode was filled with K_2SO_4, and artifacts were minimized by a special compensatory circuit. The upper traces of *A–D* were recorded first and then the micro-electrode was withdrawn from the motoneurone and the lower traces were recorded. The respective strengths of applied currents are indicated in units of 10^{-9} A, depolarizing in *A*, *B* and hyperpolarizing in *C*, *D*. In *E–H* hyperpolarizing pulses began near the start of trace and ceased near the end, the respective strengths being indicated on the records in units of 10^{-9} A. In *I–L* there were depolarizing pulses as indicated. Record *M* was response to a depolarizing pulse of 2×10^{-9} A immediately after withdrawal of the electrode to an extracellular position just outside the cell. In *N–Q* the lower trace was recorded from the filament of the ventral root containing the motor axon. With *N* and *O* the depolarizing current pulse was relatively weak; it was much stronger in *P* and *Q* and evoked a repetitive spike discharge (see text).

time course of the membrane potential change so determined would be considerably briefer than if it were determined simply by the electric time constant of the soma-dendritic membrane because electrotonic currents would be flowing to more remote regions of the dendrites (Rall, 1957; Coombs, Curtis & Eccles, unpublished observations). As would be expected, the time course of the potential changes deviates from the exponential form, there being a progressive lengthening of the successive half-times of the curve as it approaches its final equilibrium. The values of the half-time of a motoneurone have been averaged from a series of measurements along the curve in its approach almost

to 90% of its equilibrium position. The mean value of the half-time for seven motoneurones was 2·3 msec (Appendix 3, column 5).

The responses of another chromatolysed motoneurone to a wider range of currents are shown in Fig. 12*E–H* for hyperpolarizing currents and *I–L* for depolarizing. On withdrawal of the micro-electrode there was some disturbance of the coupling resistance between the two barrels, as shown by the trace, Fig. 12*M*, but the compensation remained very good for capacitive coupling, so that it can be presumed that the actual time courses of the membrane potential changes are shown in traces *E–L*. From the final steady potential change produced in the membrane by a known current, the effective membrane resistance can be calculated (cf. Coombs, Eccles & Fatt 1955*a*).

A similar investigation was carried out on several other chromatolysed motoneurones (Appendix 3, columns 5 and 8), but it was not regularly attempted because the response did not differ significantly from normal (cf. Coombs, Eccles & Fatt, 1955*a*; Frank & Fuortes, 1956; Coombs, Curtis & Eccles, 1956). When complication by partial responses was eliminated by hyperpolarization (Figs. 2–4), the mean half-time of decay of the monosynaptic EPSP of chromatolysed motoneurones was also normal with a value of 3·5 msec. (Appendix 3, column 6). In addition, the time course of the membrane response to applied current was assessed by determining the spike latencies for depolarizing currents of different strengths (cf. Frank & Fuortes, 1956). The mean calculated half-time was 1·6 msec (Appendix 3, column 7). Finally two estimates of membrane resistance (2·0 and 2·4 MΩ, Appendix 3, column 9) have been made by determining the effect of applied current in altering the size of the spike potential (cf. Frank & Fuortes, 1956). In summary it can be stated that these electrical properties of chromatolysed motoneurones do not deviate from the normal range of values.

The rheobasic current strengths for chromatolysed motoneurones (mean 5×10^{-9} A) have been generally lower than the values observed for normal motoneurones (Appendix 3, column 10). For example, mean values of $7{\cdot}4 \times 10^{-9}$ A and about 10×10^{-9} A were found by Frank & Fuortes (1956) and by Coombs, Curtis & Eccles (unpublished observations) respectively. This low value may be correlated with the propensity of chromatolysed motoneurones to give partial responses when subjected to small depolarizing currents. For example partial responses can be detected in Fig. 12*J* with a current of 6×10^{-9} A and are large in Fig. 12*K* at the rheobasic strength of 8×10^{-9} A. Doubtless such partial responses aid the depolarizing current in producing the critical level of depolarization for generating a propagated impulse, as may be seen in Fig. 12*K* and by comparison of Fig. 12*N* and *O*. Stronger depolarizing currents generated repetitive discharges of chromatolysed motoneurones (Fig. 12*P*, *Q*), possibly even more effectively than with normal motoneurones. It will be seen that the partial response in Fig. 12*N* failed to

evoke the propagation of an impulse down the motor axon (cf. Fig. 12*O*). In Fig. 12*P*, *Q* each of the successive spikes led to a propagated impulse except the last of Fig. 12*P*. The partial response at the end of *Q* also failed to propagate.

DISCUSSION

The experimental observations reported above indicate that chromatolysed motoneurones exhibit abnormal behaviour in three respects.

(1) Monosynaptic excitatory action upon them is less effective in generating the EPSP (Table 2, column 6), which rises more slowly than normal to a later summit. However, this deficiency does not lead to the expected diminution of reflex discharge because it is compensated by the abnormality next to be considered.

(2) There are exceptionally excitable patches of the soma-dendritic membrane. Small EPSP's are capable of producing partial responses of these areas, which in turn add to the depolarization of the EPSP, so compensating for its deficiency. Evidence has been given above which suggests that there are two main types of partial response: those with a steeply rising phase and an all-or-nothing behaviour, which probably are full-size spikes in remote dendritic areas that are blocked in propagation towards the soma; those with a much slower rise and more variable time course and size, which probably develop in patches on the soma or proximal dendrites. Thus it is envisaged that the excitable patches are widely distributed over the soma and dendrites. There is no regularity in the production of either type of partial response by an afferent volley (Figs. 3, 4, 5, 7, 8 and 11). Moreover, the partial responses may appear at very diverse times relative to the EPSP (Figs. 7, 8) and so at very diverse levels of depolarization. Thus it has to be postulated that the excitable patches are continually varying in the degree of their excitability, both absolutely and relatively to one another, as is well illustrated in Fig. 7*E–J*. This increased excitability of the soma-dendritic membrane is revealed also by analysis of the antidromic spike potential. The threshold level of depolarization for the antidromic generation of a soma-dendritic spike has a mean value of only 14·5 mV (Appendix 1, column 8), whereas normally the mean value has been found to be 26 mV (Coombs, Curtis & Eccles, 1957*b*).

(3) Chromatolysed motoneurones are also abnormal in respect of the responses of the initial segment of the axon. The size of the spike response (IS spike) as recorded by an intrasomatic electrode is abnormally small (Appendix 1, column 7) and antidromic propagation from the medullated axon into the initial segment has a lowered safety factor (Appendix 1, column 10).

On the basis of these three abnormalities it is possible to explain all the disordered behaviour of chromatolysed motoneurones. The most characteristic features of the reflex responses of chromatolysed motoneurones are the lengthening of the minimum latency and the great increase of temporal

dispersion of the reflex responses evoked by a group I a afferent volley. Normally the EPSP of some motoneurones rises so steeply that the depolarization reaches the threshold (about 10 mV) for initiating a reflex discharge in as brief a time as 0·4 msec from its onset. In the chromatolysed motoneurone the EPSP rises much less steeply, and, at a relatively low level of depolarization, partial responses are initiated at the exceptionally excitable patches of the soma-dendritic membrane. These partial responses add to the depolarization of the EPSP and so eventually a full-size impulse may be initiated and propagated down the axon. As is well illustrated in Figs. 7*F*, *H*, *J* and 8*H*, the additional time involved in this intermediate phase of partial responses accounts for the lengthened synaptic delay. Furthermore, there is a large degree of variability in the initiation of the partial responses, there being often a demonstrable spread from one partial response to another before the threshold depolarization was attained for initiating a propagated impulse (Fig. 7*F*, *H*, *J*); hence an explanation is provided for the large amount of temporal dispersion of the reflex discharge evoked by a group I afferent volley (cf. Fig. 8*A–D*; Downman *et al.* 1953; Bradley *et al.* 1955).

The spike-like type of local response sometimes occurred very late on the EPSP. Unfortunately the events occurring at the presumed site of initiation far out on the dendrites are not observable with a micro-electrode located intrasomatically. However, it may be assumed that such full-size impulses out in the dendrites are initiated by the same process of progressive depolarization by build-up of successive partial responses that has been observed to occur with the partial responses arising in the soma region.

Tauc (1955, 1956, 1957) has shown that, when depolarized, the ganglion cells of *Aplysia* and of *Helix pomatia* often respond by small spike-like responses that he attributes to specially excitable patches on the surface membrane, which thus would resemble the soma-dendritic membrane of chromatolysed motoneurones. Furthermore, he has postulated that the depolarization produced by activation of these excitable patches is instrumental in activating other patches and so eventually in giving the full-size spike. Thus a chromatolysed motoneurone appears to behave very similarly to a normal ganglion cell of *Aplysia* and *Helix pomatia*. Small spike responses superimposed on the excitatory post-synaptic potential have been observed by Bullock & Hagiwara (1957) when there was failure of transmission of high-frequency impulses across the giant synapse of *Loligo*.

Chromatolysed motoneurones have often resembled normal motoneurones (Coombs, Curtis & Eccles, 1957*b*) in that the actual site of initiation of the propagating impulse is either in the initial segment of the motor axon or at the first node of the medullated axon (Figs. 8*F*, 9*K*). On account of its abnormally low threshold the soma-dendritic membrane is invaded by the impulse in the initial segment after an abnormally brief delay (0·05–0·12 msec). However,

in a number of synaptically stimulated motoneurones it was impossible to detect an IS spike preceding the SD spike (Figs. 6*F*, *G*; 9*D*, *F*). Apparently the impulse was being initiated in the soma-dendritic membrane and secondarily invaded the initial segment. Such a sequence of SD–IS involvement would not be directly demonstrable as an SD spike followed by an IS spike unless the micro-electrode was in the initial segment, as appeared to be the case in the experiment illustrated in Fig. 10.

In general agreement with Bradley *et al.* (1955), chromatolysed motoneurones have not significantly differed from normal motoneurones in respect of resting potential, spike potential and the after-potentials (Fig. 1). There was also no appreciable abnormality in respect of the resistance and electric time constant of the motoneuronal membrane (Appendix 3), so the membrane capacity also would be within the normal range. It can therefore be concluded that the diminution of the monosynaptic EPSP (Appendix 2, column 6), is due to a diminished effectiveness of the excitatory synaptic knobs, though the latency of their action is within normal limits (Appendix 2, column 5). Brown & Pascoe (1954) likewise found that there was a depression of excitatory synaptic action on sympathetic ganglion cells that had had their axons severed about 3 weeks previously. They made the further important observation that on presynaptic excitation the output of acetylcholine was undiminished, but that the ganglion cells were less sensitive to acetylcholine. It therefore seems likely that the diminished EPSP's of chromatolysed motoneurones are due to a diminished effectiveness of the synaptic transmitter, but the testing of this postulate must await the identification of the synaptic transmitter substance.

There is insufficient evidence with respect to the effectiveness of the inhibitory synaptic knobs, but they appear to resemble the excitatory knobs in having a lengthened time of action, as indicated by the longer time to maximum of both the EPSP (Appendix 2, column 9) and the IPSP produced by relatively synchronous synaptic bombardments.

Eventually it would be desirable to correlate the functional changes with the histological abnormalities of chromatolysed motoneurones. Such a correlation must await a thorough study of chromatolysed motoneurones by electron microscopy. However, even such refined procedures may fail to reveal the changes in micro-structure of the synapses and the various components of the motoneurone which are responsible for the disordered functions as outlined above.

Normally the specially low threshold of the initial segment of a motoneurone ensures that synaptic excitatory action always causes the initiation of an impulse there (or at the first node of the myelinated axon) and never primarily in the soma-dendritic membrane (Eccles, 1957; Coombs, Curtis & Eccles, 1957*a*, *b*). The soma-dendritic membrane functions essentially in generating the excitatory and inhibitory synaptic potentials and in transmitting

them electrotonically to the initial segment of the motor axon where the impulse is initiated. Thus the low threshold of the initial segment relative to the soma-dendritic membrane adapts a motoneurone particularly well for its function of integrating all its diverse inhibitory and excitatory synaptic bombardment, and so acting efficiently as a final common path. On account of the change in relative thresholds, the chromatolysed motoneurone is a defective integrator, for a partial response can now be generated in a dendrite by a special strategic grouping of excitatory synapses (cf. Fig. 11 *C*, *D*). This partial response could grow to such a size as to develop a propagated impulse either in the dendrite-soma region or in the initial segment of the axon, despite powerful inhibitory action elsewhere on that motoneurone.

Downman *et al.* (1953) mistakenly postulated that the delayed and prolonged reflex discharges evoked by group I afferent volleys were due to the action of polysynaptic pathways that had been developed in relationship to the chromatolysed motoneurones. The development of polysynaptic pathways was also suggested by the increase in reflex discharges evoked by cutaneous afferent volleys that normally operate polysynaptically. Such an increase would now also be explicable by the specially excitable patches on the soma-dendritic membrane. However the possibility of new growth of polysynaptic paths must not be excluded, particularly as there is now evidence that this occurs under other conditions in the mammalian spinal cord (McCouch, Austin & Liu, 1955). In our experiments there has been some evidence for the development of abnormal excitatory connexions in two of the three spinal cords that had specially long periods of chromatolysis (27 and 44 days). Further investigation is desirable.

SUMMARY

1. The responses of chromatolysed motoneurones of cats have been studied by intracellular recording from 14 to 44 days after the appropriate ventral roots have been severed extradurally; i.e. during a period when the characteristic histological changes are most prominent.

2. Chromatolysed motoneurones have not significantly differed from normal motoneurones in respect of resting potential, spike potential or after-potentials. Also inhibitory synaptic potentials were changed only in respect of a slight lengthening of rising phase. The principal abnormalities appear in relation to the potentials which are generated by excitatory synaptic action (the EPSP's) and in the manner of initiation of impulses by the motoneurone.

3. The monosynaptic EPSP's are significantly reduced in size and in time to summit, and superimposed upon them are a variety of hump-like depolarizations which are transitional to the generation of full-size impulses with the discharge of impulses down the motor axons. Hyperpolarization of a motoneurone reversibly depresses or abolishes these humps, which consequently are attributed either to responses of patches of the soma-dendritic membrane

or to full-size impulses restricted to remote dendritic areas. The former responses show an extreme variability of size and time course, whereas the latter are like small spikes and have an all-or-nothing character.

4. It is inferred that there are abnormally excitable patches of membrane on the soma and dendrites, and it is shown that the threshold level of depolarization for spike initiation is abnormally low for the soma-dendritic membrane.

5. Chromatolysed motoneurones are also abnormal in that antidromic propagation into the initial segment has a lowered safety factor and its spike potential (the IS spike) is small relative to the SD spike.

6. It is shown that these abnormalities account for the longer latency and greatly increased temporal dispersion of the reflex discharge evoked by a group I afferent volley.

7. The unfavourable effect on the integrative function of the motoneurone is discussed.

8. There has been no satisfactory evidence that new polysynaptic pathways are formed to chromatolysed motoneurones.

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Appendix 1

Antidromic propagation into chromatolysed motoneurones

1 Cell type	2 Days chromat.	3 Rest. potential (mV)	4 M spike (mV)	5 M–IS interval (msec)	6 IS–SD interval (msec)	7 IS/SD slopes	8 Thresh. SD (mV)	9 SD spike (mV)	10 Min. A_1A_2 interval IS (msec)	11 Min. A_1A_2 interval SD (msec)
Pl	22	−60	4·5	0·20	0·14	0·30	18	94	—	—
?	22	−80	2·6	0·22	0·14	0·30	13	100	—	—
BST	22	−60	3·3	0·07	0·17	0·28	12	76	—	—
?	22	−75	4·4	0·07	0·18	0·30	16	84	1·5	2·2
BST	21	−63	2·4	0·10	0·15	0·34	12	80	2·5	2·5
FDL	21	−65	2·7	0·22	0·14	0·30	11	76	—	—
FDL	21	−66	2·3	—	—	0·30	15	87	2·5	2·5
Pl	20	−60	2·7	—	0·15	0·23	16	83	55	55
DP	16	−50	—	0·17	0·13	0·25	12	77	—	—
Pl	16	−60	2·5	0·05	0·11	0·30	15	78	15	15
?	16	−50	—	0·05	0·10	0·30	13	77	3·7	3·7
?	16	−52	—	—	0·14	0·20	13	76	2·4–4·9	2·4–4·9
FDL	16	—	3·5	0·11	0·11	0·26	19	88	2·7	2·7
Pl	14	−50	—	—	0·13	0·33	16	83	3·9	3·9
Pl	14	−70	3·2	0·21	0·13	0·32	17	87	—	—
GS	14	−65	—	—	0·15	0·35	15	78	1·4	1·4
BST	14	—	—	—	0·15	0·40	18	97	1·8	2·2
GS	14	−60	—	—	0·15	0·30	10	73	1·5	1·7
Means		−62	3·1	0·13	0·14	0·30	14·5	83	—	—

Throughout appendices: BST = biceps-semitendinosus; FDL = flexor digitorum longus; GS = gastrocnemius-soleus; Pl = plantaris; DP = deep peroneal group of muscles.

Appendix 2

The excitatory post-synaptic potential and the latency of its initiation of spikes in chromatolysed motoneurones

1 Cell type	2 Days chromat.	3 Rest. potential (mV)	4 SD spike (mV)	5 Latency EPSP (msec)	6 Max. slope EPSP (V/sec)	7* Min. latency spike (msec)	8* Max. latency spike (msec)	9* Time to summit of EPSP (msec)
GS	44	−63	70	0·6	6	1·4	3·3	1·9
BST	23	−70	70	0·7	6	—	4·0	3·1
Pl	22	−60	94	0·6	9	1·5	2·6	2·4
BST	22	−60	76	0·5	9	1·5	5·0	—
BST	21	−63	80	0·6	7	1·3	3·2	2·6
Pl	20	−60	83	0·5	1·0	1·7	2·5	2·0
GS	20	−60	88	0·6	8	2·0	7·1	2·1
DP	16	−50	77	0·65	2	1·3	5·8	2·25
Pl	16	−60	78	0·5	2·5	—	—	—
FDL	16	—	88	0·6	5·5	1·45	4·0	2·5
BST	16	−55	73	0·6	3	1·6	8·1	2·75
GS	16	−55	70	0·6	7	1·7	3·7	2·2
Pl	14	−50	83	0·6	5	2·3	9·0	3·0
Pl	14	−60	87	0·6	5	1·75	8·5	2·75
GS	14	−65	78	0·7	6	1·7	3·1	2·5
GS	14	−60	73	0·7	5	2·3	3·8	2·5
Means		−59	79	0·6	5·5	1·7	—	2·4

* The times were measured from the entry of the afferent volley into the spinal cord. Column 9 gives values after the partial responses were suppressed by hyperpolarization.

APPENDIX 3

Electrical properties of the surface membrane of chromatolysed motoneurones

1 Cell type	2 Days chromat.	3 Rest. potential (mV)	4 SD spike (mV)	5 Memb. half-time (msec)	6 EPSP decay half-time (msec)	7 Strength latency half-time (msec)	2 Memb. resist. (direct measure-ment) (MΩ)	9 Memb. resist. (spike potential measure-ment) (MΩ)	10 Rheo-basic current ($\times 10^{-9}$A)
BST	23	−60	63	3·3	4·8	—	1·9	—	4
BST	22	−60	76	2·4	—	1·15	1·3	—	2
Pl	20	−60	83	—	3·3	2·5	—	2·0	8
BST	20	−46	67	2·0	2·4	—	1·1	—	4
GS	20	−60	88	3·4	4·2	1·7	2·5	2·4	6
BST	20	−55	63	1·4	2·9	—	2·3	—	6
?	16	−48	67	1·3	—	0·9	0·8	—	5
Pl	16	−60	78	2·4	3·1	1·7	1·3	—	4
Means		−56	73	2·3	3·5	1·6	1·6	—	5